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DOCUMENT-IDENTIFIER: US 6080400 A
TITLE: Compositions for the prevention and treatment of verotoxin-induced disease

DEPR:
As used herein, the term "neutralizing" is used in reference to antitoxins, particularly antitoxins comprising antibodies, which have the ability to prevent the pathological actions of the toxin against which the antitoxin is directed. It is contemplated that neutralizing antibodies be utilized to prevent the action of toxins, in particular E. coli verotoxins and Shiga toxin. It is further contemplated that neutralizing antibodies be utilized to alleviate the effect(s) of toxins in an individual, in particular E. coli verotoxins and Shiga toxin.

DEPR:
As used herein, the term "immunogen" refers to a substance, compound, molecule, or other moiety which stimulates the production of an immune response. The term "antigen" refers to a substance, compound, molecule, or other moiety that is capable of reacting with products of the immune response. For example, verotoxin subunits may be used as immunogens to elicit an immune response in an animal to produce antibodies directed against the subunit used as an immunogen. The subunit may then be used as an antigen in an assay to detect the presence of anti-verotoxin subunit antibodies in the serum of the immunized animal.

DEPR:
The term "monovalent" when used in reference to a verotoxin vaccine refers to a vaccine which is capable of provoking an immune response in a host animal directed against a single type of verotoxin. For example, if immunization of a host with E. coli VT1 toxin vaccine induces antibodies in the immunized host which protect against a challenge with VT1, but not against challenge with other toxins (e.g., VT2), then the VT1 vaccine is said to be monovalent. In contrast, a "multivalent" vaccine provokes an immune response in a host animal directed against more than one verotoxin. For example, if immunization of a host with a vaccine comprising VT1 and VT2 verotoxins induces the production of antibodies which protect (i.e., "protective antibody") the host against a challenge with both VT1 and VT2, the vaccine is said to be multivalent (in particular, this hypothetical vaccine is bivalent). It is intended that multivalent vaccines of the present invention encompass numerous embodiments. For example, it is also contemplated that recombinant E. coli verotoxin proteins be used in conjunction with either native toxins or toxoids from

other organisms as antigens in a multivalent vaccine preparation. It is further contemplated that multivalent vaccines of the present invention will stimulate an immune response against various E. coli serotypes, including, but not limited to E. coli O157:H7, O216:H11, O113:H21, O91:H21, and O111:NM, in humans and/or other animals.

DEPR:
The present invention also provides methods of generating neutralizing antibody directed against Escherichia coli verotoxin comprising: providing in any order: an antigen comprising a fusion protein comprising a non-toxin protein sequence and at least a portion of a Escherichia coli verotoxin, the toxin selected from the group consisting of type 1 toxin and type 2 toxin, a host; and immunizing the host with the antigen so as to generate a neutralizing antibody.

DEPR:
The first set of Examples (Examples 1-5) was designed to develop an antidote to E. coli O157:H7 verotoxins and evaluate its effectiveness in vitro and in vivo. In the first experiments, high titer verotoxin antibodies were generated in laying hens hyperimmunized with chemically detoxified and/or native verotoxins. These Laying hens were immunized with either recombinant E. coli O157:H7 VT1 or VT2 (rVT1 and rVT2) treated with glutaraldehyde and mixed with adjuvant.

DEPR:
Fourth, VT neutralization potency was analyzed in vitro using a Vero cytotoxicity assay. Vero cytotoxicity of rVT1 and rVT2 could be completely inhibited by VT IgY. These antibodies also demonstrated substantial verotoxin cross-neutralization.

DEPR:
Fifth, the efficacy of passively administered avian verotoxin antibodies in preventing the lethal effects of verotoxin poisoning was assessed in a mouse disease model. Toxin neutralizing antibodies were administered by parenteral dosing regimens to assess the most effective strategy for therapeutic intervention. Efficacy of verotoxin antibodies was demonstrated using multiple murine disease models. In these experiments, antibodies prevented both the morbidity and lethality of homologous and heterologous toxins using a toxin/antitoxin premix format; mice infected orally with a lethal dose of viable E. coli O157:H7 were protected from both morbidity and lethality when treated parenterally four hours post-infection with either rVT1 or rVT2 antibodies; and mice given a lethal dose of E. coli O91:H21 (a particularly virulent strain which only produces VT2c, a VT2 structural variant) and treated parenterally up to 10 hours later with rVT1 IgY administered parenterally were protected from both morbidity and lethality.

DEPR:
Laying Leghorn hens were immunized as described above (Example 1, part E), using glutaraldehyde-treated rVT's. Following several immunizations, eggs were collected and IgY harvested by PEG fractionation. FIGS. 4 and 5 show rVT1 or rVT2 specific antibody

responses detected using EIA at dilutions of the original yolk IgY concentration of 1:30,000 and 1:6,000, respectively. IgY fractionated similarly from unimmunized hens (i.e., preimmune antibody) did not react with either antigen at test dilutions above 1:50. Although these EIA results indicate significant antibody responses, prior experience with other toxin antigens has shown that optimization of immunization regimens, including increasing the amount of antigen, can yield titers in excess of 1:100,000 (B. S. Thalley, et al.,"Development of an Avian Antitoxin to Type A Botulinum Neurotoxin," in Botulinum and Tetanus Neurotoxins: Neurotransmission and Biomedical Aspects, B. R. DasGupta, (ed.) [Plenum Press, New York, 1993] pp. 467-472). As may be expected due to their structural homology and consistent with previous reports (e.g., V. V. Padhye et al., "Production and characterization of monoclonal antibodies to verotoxins 1 and 2 from Escherichia coli O157:H7," J. Agr. Food Chem., 39: 141-145 [1989]; S. C. Head et al., "Purification and characterization of verocytotoxin 2," FEMS Microbiol. Lett., 51: 211-216 [1988]; and N. C. Strockbine et al., "Characterization of Monoclonal Antibodies against Shiga-Like Toxin from Escherichia coli," Infect. Immun., 50: 695-700 [1985]), FIGS. 4 and 5 also demonstrate that antibodies generated against one toxin cross-reacted in vitro with the other toxin.

DEPR:
To assess the broader utility of the IgY verotoxin antibodies in treating VTEC-associated disease, the mouse infection study was performed using a more virulent VTEC serotype known to produce VT2c--a structural variant of VT2--but not VT1 (S. W. Lindgren et al., "Virulence of enterohemorrhagic Escherichia coli O91:H21 clinical isolates in an orally infected mouse model," Infect. Immun., 61: 3832-3842 [1993]).

DEPR:
The results from these Examples clearly demonstrate the feasibility and provide the experimental basis for development of an avian antidote for E. coli O157:H7 verotoxins suitable for use in humans. In contrast to previous reports showing that rabbit polyclonal VT1 and VT2 antibodies cross-reacted, but did not cross-neutralize the heterologous toxin in Vero cytotoxicity or in mouse lethality studies (e.g., V. V. Padhye et al., "Production and characterization of monoclonal antibodies to verotoxins 1 and 2 from Escherichia coli O157:H7," J. Agr. Food Chem., 39: 141-145 [1989]; S. C. Head et al., "Purification and characterization of verocytotoxin 2," FEMS Microbiol. Lett., 51: 211-216 [1988]; and N. C. Strockbine et al., "Characterization of monoclonal antibodies against Shiga-like toxin from Escherichia coli," Infect. Immun., 50: 695-700 [1985]), these data provide the first demonstration of cross-neutralization in vivo. Antibodies against one toxin neutralized completely the heterologous toxin in both Vero cytotoxicity and mouse lethality assays. Both rVT1 and rVT2 antibodies also prevented morbidity (as assessed by renal histopathology) and mortality in mice infected with lethal doses of E. coli O157:H7--the etiologic agent in 90% of the documented cases of hemolytic uremic syndrome (HUS) in the U.S. (P. M. Griffin and R. V. Tauxe, "The epidemiology of infections caused by Escherichia coli O157:H7,

other enterohemorrhagic E. coli, and the associated hemolytic uremic syndrome," Epidemiol. Rev., 13: 60 [1991]). With at least two other VTEC serotypes known to cause HUS, the finding that rVT1 antibodies neutralized a VT2 variant produced by E. coli O91:H21 suggests that avian polyclonal antibodies may provide an effective antidote against other verotoxin-producing E. coli. These data also show for the first time, that antibodies may be administered after infection and still protect against morbidity and mortality.